Medicine in focus

Ebola virus: The role of macrophages and dendritic cells in the pathogenesis of Ebola hemorrhagic fever

Mike Bray [a,*], Thomas W. Geisbert [b]

[a] *Biodefense Clinical Research Branch, Office of Clinical Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA*

[b] *Virology Division, US Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, MD 21702-5011, USA*

Abstract

Ebola hemorrhagic fever is a severe viral infection characterized by fever, shock and coagulation defects. Recent studies in macaques show that major features of illness are caused by effects of viral replication on macrophages and dendritic cells. Infected macrophages produce proinflammatory cytokines, chemokines and tissue factor, attracting additional target cells and inducing vasodilatation, increased vascular permeability and disseminated intravascular coagulation. However, they cannot restrict viral replication, possibly because of suppression of interferon responses. Infected dendritic cells also secrete proinflammatory mediators, but cannot initiate antigen-specific responses. In consequence, virus disseminates to these and other cell types throughout the body, causing multifocal necrosis and a syndrome resembling septic shock. Massive "bystander" apoptosis of natural killer and T cells further impairs immunity. These findings suggest that modifying host responses would be an effective therapeutic strategy, and treatment of infected macaques with a tissue-factor inhibitor reduced both inflammation and viral replication and improved survival.
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Keywords: Ebola virus; Ebolavirus; Filovirus; Disseminated intravascular coagulation; Septic shock; Therapy

1. Introduction

Ebola hemorrhagic fever (EHF) is one of the most severe viral infections of humans. In outbreaks in central Africa caused by the *Zaire* species of *ebolavirus* (ZEBOV), the mortality rate among identified cases has reached 80–90%, while fatalities in epidemics caused by the Sudan species have been in the range of 50–60% (Bwaka et al., 1999; Sanchez et al., 2004). The natural reservoir of these agents has not been identified; humans are only accidental or "dead-end" hosts (Mahanty & Bray, 2004).

EHF begins with the abrupt onset of fever and malaise, followed over several days by a fall in blood pressure leading to profound shock and the develop-

* Corresponding author. Tel.: +1 301 451 5123;
fax: +1 301 480 2319.
E-mail addresses: mbray@niaid.nih.gov (M. Bray),
tom.geisbert@amedd.army.mil (T.W. Geisbert).

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14. ABSTRACT

Ebola hemorrhagic fever is a severe viral infection characterized by fever, shock and coagulation defects. Recent studies in macaques show that major features of illness are caused by effects of viral replication on microphages and dendritic cells. Infected macrophages produce proinflammatory cytokines, chemokines and tissue factor, attracting additional target cells and inducing vasodilation, increased vascular permeability and disseminated intravascular coagulation. However, they cannot restrict viral replication, possibly because of suppression of interferon responses. In consequence, virus disseminates to these and other cell types throughout the body, causing multifocal necrosis and a syndrome resembling septic shock. Massive "bystander" apoptosis of natural killer and T cells further impairs immunity. These findings suggest that modifying host responses would be an effective therapeutic strategy, and treatment of infected macaques with a tissue-factor inhibitor reduced both inflammation and viral replication and improved survival.

15. SUBJECT TERMS
filovirus, Ebola virus, disseminated intravascular coagulation, DIC, septic shock, therapy

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ment of severe coagulation defects. In some patients, antigen-specific immune responses develop in time to restrict viral replication and bring about survival, otherwise death occurs 1–2 weeks after the onset of symptoms (Sanchez et al., 2004). No anti-viral drugs have been identified that block *ebolavirus* replication. Patient care is supportive in nature.

This article focuses on the pathogenesis of EHF caused by ZEBOV, the viral species that has caused the largest number of outbreaks in Africa and has been studied most extensively in the laboratory. Because human clinical studies have yielded only fragmentary, often contradictory information, this article principally summarizes data obtained from recent laboratory studies of the uniformly lethal disease caused by ZEBOV in cynomolgus and rhesus macaques. Features of illness seen in fatal human cases include fever, a high circulating viral load, a marked rise in blood neutrophil count and fall in lymphocytes and platelets, hypotension and shock, coagulopathy and hemorrhage, and biochemical alterations suggestive of massive lymphocyte apoptosis (Bwaka et al., 1999; Baize et al., 1999; Sanchez et al., 2004; Towner et al., 2004). All of these changes are also seen in ZEBOV-infected macaques. The coagulopathy in macaques conforms to the definition of disseminated intravascular coagulation (DIC), but this has not yet been proven to occur in humans (Geisbert, Hensley, Larsen et al., 2003; Geisbert, Young, Jahrling, Davis, Kagan et al., 2003; Geisbert, Young, Jahrling, Davis, Larsen et al., 2003).

2. Overview of pathogenesis

ZEBOV is a nonsegmented, negative-strand virus in the family *Filoviridae* (Fig. 1). Ebola virions are able to infect a broad range of primate cells, perhaps because the heavily glycosylated surface glycoprotein (GP) can bind to a variety of target molecules, including cell-surface lectins (Takada et al., 2004). Replication results in necrosis of infected cells.

Studies in macaques have demonstrated that the major early targets of ZEBOV infection are two types of cells: macrophages, which employ a battery of innate immune mechanisms for initial anti-viral defense, and dendritic cells (DC), which have innate immune functions, but also specialize in initiating adaptive immune responses by presenting antigens to naïve T cells

(Fig. 2). ZEBOV infection partially impairs the function of both cells, so that they are able to initiate inflammation and coagulation, but cannot prevent the systemic spread of virus. In consequence, additional target cells are attracted to sites of infection, and virus disseminates to resident macrophages and DC in tissues throughout the body, causing massive release of proinflammatory mediators and vasoactive substances (Hensley, Young, Jahrling, & Geisbert, 2002). These host responses produce a syndrome of refractory hypotension and DIC resembling septic shock, which results from the response of the same cell populations to endotoxin and other bacterial products (Bray & Mahanty, 2003). The extensive tissue injury caused by replication of ZEBOV in macrophages and DC and in parenchymal cells of the liver and other organs also plays a major role in fatal disease (Fig. 2). Natural killer (NK) cells and T lymphocytes remain uninfected, but undergo apoptosis, further impairing immune function (Geisbert et al., 2000; Geisbert, Hensley, Larsen et al., 2003; Reed, Hensley, Geisbert, Jahrling, & Geisbert, 2004).

3. ZEBOV effects on macrophage function

Macrophages play a central role in inducing the hypotension and shock of EHF (Fig. 2). The binding of double-stranded RNA or other viral products to pattern-recognition molecules triggers cytoplasmic-signalling pathways that bring about the migration of NF-κB and other transcriptional activators to the nucleus, resulting in release of proinflammatory cytokines, such as TNF-α and IL-1β, chemokines, such as MIP-1α, and nitric oxide (NO) and other vasoactive molecules (Gupta, Mahanty, Ahmed, & Rollin, 2001; Hensley et al., 2002; Stroher et al., 2001). These mediators attract additional monocytes/macrophages to the site of infection, mobilize immature neutrophils from blood vessel walls and the bone marrow and facilitate the exit of inflammatory cells and proteins from the circulation by causing vasodilatation, increased endothelial permeability and expression of endothelial cell-surface adhesion molecules. Although these changes in vascular function may be beneficial in resolving a localized infectious lesion, their occurrence throughout the body as a result of the systemic spread of ZEBOV leads to catastrophic circulatory collapse (Bray & Mahanty,
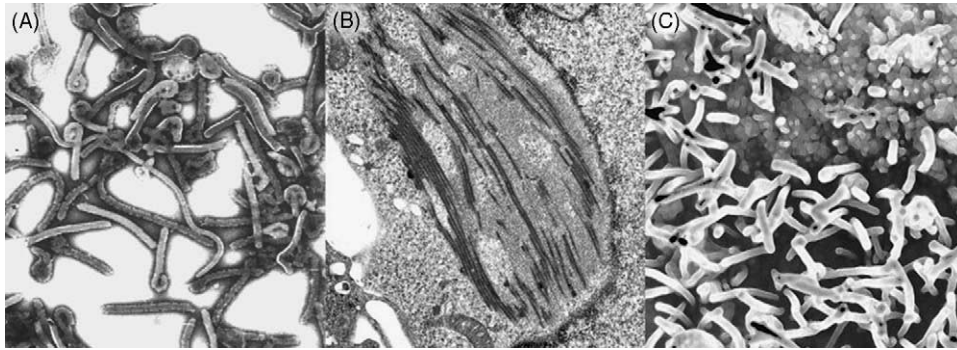
Fig. 1. (A) Transmission electron micrograph (TEM) showing the characteristic filamentous structure of ZEBOV virions. Each contains a negative-sense RNA genome and all enzymes and factors required for genome replication and transcription of viral genes. (B) Binding of virions to the cell surface is followed by membrane fusion within endosomes and release of the genome and associated proteins into the cytoplasm. A four-protein replication complex then generates positive-sense "anti-genomes" that serve as templates for transcription of messenger RNA encoding the viral proteins. The viral genome encodes a truncated, secreted form of the virion GP; a full-length membrane-bound form is generated through transcriptional "editing" (Sanchez et al., 1998). The figure shows nascent nucleocapsids aggregated in cytoplasmic inclusion bodies in an infected hepatocyte (TEM). (C) New virions form, when nucleocapsids associate with viral matrix proteins and the cytoplasmic tails of GP molecules embedded in the cell membrane. Nascent ZEBOV virions are shown budding through the surface of an infected primary human endothelial cell (SEM).

2003; Geisbert, Young, Jahrling, Davis, Larsen et al., 2003; Mahanty & Bray, 2004).

Virus-infected macrophages also play an important role in initiating DIC by synthesizing cell-surface tissue factor (TF), which interacts with circulating factors VIIa and X to trigger the extrinsic coagulation pathway, leading to deposition of fibrin on the surface of infected cells and on membrane microparticles released into the bloodstream (Geisbert, Young, Jahrling, Davis, Kagan et al., 2003). Binding of coagulation factors to cell-surface TF also alters macrophage function by exciting intracellular signalling pathways, through the phosphorylation of the cytoplasmic tails of TF and associated membrane-bound protease-activated receptors (PARs) (Ruf, 2004). Additional factors contributing to severe coagulopathy may include the release of additional TF in areas of necrosis, increased initiation of clotting on altered endothelial surfaces, and the release of fibrin degradation products, such as D-dimers, into the plasma as thrombi are broken down by plasmin and other enzymes. In ZEBOV-infected macaques, D-dimers are detectable on the first day postinfection (Geisbert, Young, Jahrling, Davis, Kagan et al., 2003). Thrombocytopenia, by contrast, does not become evident until days 3–4, as platelets attach to activated endothelium or become part of nascent thrombi.

The ability of ZEBOV to disseminate rapidly from its site of entry suggests that infected cells are unable to produce sufficient amounts of interferon (IFN)-α/β or respond adequately to exogenous types I or II IFN. There is good evidence that the ZEBOV VP35 protein blocks IFN production by virus-infected cells in a manner similar to the influenza virus NS1 protein, by preventing the recognition of dsRNA that normally leads to phosphorylation of IRF-3 and its translocation to the nucleus (Basler and Palese, 2004; Hartman, Towner, & Nichol, 2004). Preliminary findings suggest that a second viral protein, VP24, contributes to this process by blocking responses to exogenous IFN (Basler and Palese, 2004). Such inhibition would profoundly impair anti-viral defenses, since types I and II IFN are needed to activate NK cells, assist adaptive immunity through upregulation of major histocompatibility complex (MHC) molecules and activate macrophages and DC for effective anti-microbial function.

4. Effects on dendritic cell function

Since DC play a critical role as "gatekeepers" in the induction of antigen-specific immunity, their response to ZEBOV infection may be crucial in determining the outcome of infection. Inhibition of DC function
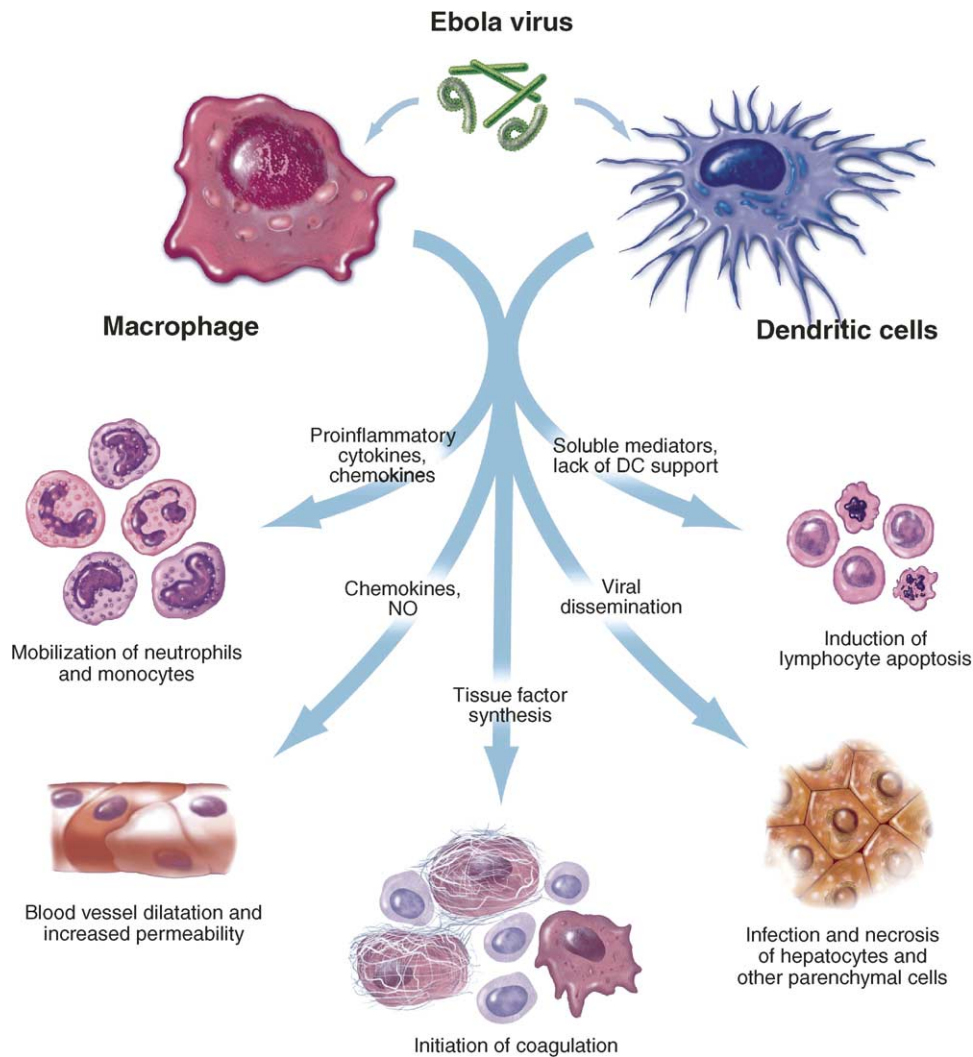
Fig. 2. ZEBOV-infected macrophages and DC play a central role in inducing the clinical features of Ebola hemorrhagic fever. Secreted cytokines, chemokines and other mediators alter blood vessel function and elicit an influx of inflammatory cells, including additional monocytes/macrophages, to sites of infection, while the synthesis of cell-surface tissue factor contributes to systemic coagulopathy. Virus released from infected macrophages and DC spreads to similar cells throughout the body and to parenchymal cells in many organs, resulting in multifocal tissue necrosis. The ability of the host to develop an effective adaptive immune response is weakened by massive lymphocyte apoptosis, a phenomenon also seen in bacterial sepsis (Hotchkiss et al., 2003).

by ZEBOV has been demonstrated by comparing the responses of human myeloid DC to noninfectious virus-like particles (VLP) or to live virus. Exposure of immature DC to VLP triggered a strong inflammatory response, with release of TNF-α, IL-6, IL-8, and MIP-1α, and induced their transformation to an antigen-presenting phenotype by upregulating costimulatory molecules CD40, CD80, and CD86, MHC classes I and

II surface antigens, downregulating chemokine receptor CCR5 and upregulating CCR7 (Bosio et al., 2004). The activated cells were able to induce proliferation of naïve lymphocytes. By contrast, ZEBOV-infected myeloid DC secreted only a limited range of chemokines (Fig. 2), failed to express costimulatory molecules or upregulate MHC and were unable to induce differentiation of allogenic lymphocytes (Bosio

et al., 2003; Geisbert, Hensley, Larsen et al., 2003; Mahanty et al., 2003). Additional research is needed to examine the effect of ZEBOV infection on plasmacytoid DC, which plays an important part in controlling viral infections by secreting large amounts of type I IFN.

5. Induction of lymphocyte apoptosis

Even though ZEBOV does not replicate in lymphocytes, large numbers of these cells undergo apoptosis in infected macaques, explaining the progressive lymphopenia observed over the course of illness (Geisbert et al., 2000; Reed et al., 2004). Blood samples from fatally infected African patients also show reduced lymphocyte counts and biochemical markers of apoptosis, suggesting that a similar process occurs in humans (Baize et al., 1999).

Like coagulation abnormalities, lymphocyte apoptosis begins early in infection. A number of mediators produced by virus-infected macrophages, including TNF-α, Fas and its ligand, TNF-α-related apoptosis-inducing ligand (TRAIL) and NO, may be capable of inducing apoptosis (Hensley et al., 2002; Reed et al., 2004). Impaired DC function may also contribute to the elimination of lymphocytes. Recent studies indicate that NK cells and CD4+ and CD8+ lymphocytes are the principal cell types affected in ZEBOV-infected macaques. Since they are important sources of IFN-γ, their early loss may prevent the activation of macrophages and other inflammatory cells needed to restrict viral replication. Lymphopenia is also seen in other viral hemorrhagic fevers, and a massive apoptotic loss of lymphocytes occurs in septic shock, suggesting that similar host responses occur in these conditions (Hotchkiss & Karl, 2003; Hotchkiss, Tinsley, & Karl, 2003).

6. Outcome of ZEBOV infection in nonhuman primates and humans

Because primates are only accidental hosts for ZEBOV, there has been no opportunity for the evolution of effective defenses against the virus, and some responses to infection appear to be inappropriate or even damaging to the host. Laboratory infection of macaques provides a "worst case scenario", since even very small doses of ZEBOV cause uniformly lethal infection, when injected, delivered by aerosol or placed in the mouth or on the conjunctiva (Johnson, Jaax, White, & Jahrling, 1995; Jaax et al., 1996). By contrast, some 10–20% of ZEBOV-infected humans in African outbreaks begin to show clinical improvement during the second week of illness and recover from their infection. Survival appears to correlate with the development of an antigen-specific immune response, usually marked by the appearance of ZEBOV-specific IgG (Baize et al., 1999; Sanchez et al., 2004).

This difference in outcome may simply reflect the disparity between controlled infection in the laboratory and the varied exposures that occur under natural conditions, but it may also indicate genetically determined differences in host responses to ZEBOV infection, both between humans and macaques and among members of the human population. For example, ZEBOV infection of macaques results in a continuing increase in circulating proinflammatory cytokines over the course of illness, in the absence of anti-inflammatory mediators, such as IL-10, while blood samples from human cases have shown the presence of both proinflammatory cytokines, such as TNF-α and IL-6, and anti-inflammatory mediators, such as IL-10 and IL-1β receptor antagonist (Baize et al., 1999, 2002). As in the case of septic shock, fatal infection of humans appears to be associated with an elevation of anti-over-proinflammatory cytokines, suggesting that the balance and timing of early responses to infection play a critical role in determining its outcome (Bray & Mahanty, 2003).

7. Modification of host responses as a therapeutic strategy

If ZEBOV elicits damaging host responses, then therapeutic interventions that modify those responses may help the primate immune system to control viral replication and achieve survival. Two approaches of this type have proven beneficial in macaques. The first takes aim at the severe coagulopathy of EHF by employing recombinant nematode anti-coagulant protein (rNAP)c2, which blocks the interaction of TF with factor VIIa. Administration of rNAPc2 to ZEBOV-infected macaques, beginning on the day of infection,

markedly reduced physical signs and laboratory indices of DIC, prevented the death of one-third of treated animals and significantly prolonged survival of the others (Geisbert, Hensley, Jahrling et al., 2003). Unexpectedly, treatment also resulted in a marked decrease in IL-6 and MIP-1α levels and a 100-fold drop in peak viral load in survivors. These unanticipated effects of anti-coagulant therapy are evidence of an interlocking relationship among inflammation, coagulation and ZEBOV replication, and provide a potent stimulus to further research.

The second approach attempts to compensate for virus-induced suppression of IFN responses. In the only experiment in macaques reported so far, treatment with a single IFN-α subtype, recombinant human IFN-α 2b, caused a delay in death (Jahrling et al., 1999). More recent, unpublished research suggests that therapy that more closely mirrors a natural IFN response by including IFN-β and multiple subtypes of IFN-α may have a more potent protective effect. Treatment with IFN-γ might further strengthen innate antiviral responses, while adding rNAPc2 could provide a synergistic effect. The fact that modification of host responses has been effective against uniformly lethal ZEBOV infection in nonhuman primates suggests that this approach will provide even greater benefit in humans.

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